Research Article
Open Access

Isolation and identification of some pathogenic bacteria species from contaminated ambient with oily hydrocarbons

Abstract

Pathogenic species may naturally at nature in different conditions, at fields of petrol and contaminated area. The pathogenic species *Citrobacter amalonaticus* which is a genus of coliform bacteria that is a gram-negative within the family of intestinal bacteria, were isolated from water samples 3, 4, and 5 of Al-Ahdab field in addition of presence *Bacillus subtilis,* but pathogenic gram-negative species *Sphingomonas paucimobilis* bacteria found as a biodegradable organism was identified and isolated from samples of soil from a Al-Ahdad filed 1 and 2, In addition of presence *Pseudomonas aeruginosa* in a soil sample of Al-Gharaaf field at Al-Nnasiriyah in Iraq. These results were different from municipal fuel of Al-Sidiyeh station, Brother's station, and Al-Amel station for calibration, which shown just *Bacillus* spp., *B. cereus,* in general especially in polluted soil with diesel with no presence of *C. amalonaticus,* and *S. paucimobilis*. Isolation and identification of bacterial depended on morphology using triple light microscope and biochemical tests. Furthermore we can use pathogenic microorganisms in biodegradation.

Keywords: isolation, diagnosis of bacteria, contaminated soil, oil products and pollution

Volume 8 Issue 4 - 2020

Ahmed Ibrahim Jessim, Rasha Kifah Hassan, Ammar Salim Salman
Ministry of Science and Technology, Center for research and evaluation, Directorate of Treatment and disposal of chemical, biological and military hazardous wastes, Iraq

Correspondence: Ahmed Ibrahim Jessim, Ministry of Science and Technology, Center for research and evaluation, Directorate of Treatment and disposal of chemical, biological and military hazardous wastes, Iraq, Tel +9647713659713, Email ahm_jas71@yahoo.com

Received: October 08, 2020 | **Published:** October 16, 2020

Introduction

Living microorganisms content of soils are a very important part of natural ecosystems, where they can adjust the flow of energy.[1] As well as the cycle of materials by consumption of animal and plant wastes, these microorganisms play a pivotal role in the growth of agricultural plants and multiplication of agricultural crops.[2] Also they can act a positive role in ecological soil system, in addition to the bioremediation and to the transfer of organic substances.[3] Moreover, microbial diversity in the soil is related closely to the function and structure of the surrounding ecosystem.[4] In addition to that they are considered as a component of maintaining soil productivity.[5] As a result of ability of living microorganisms like bacteria, fungus, yeasts and microalgae to remove hydrocarbons complexes,[6] where bacteria are present in contaminated areas with oil derivatives and the nature of specialized bacteria sometimes found in contaminated soils, the strain of *Pseudomonas* strain PS-1 has been found in these soils due to their ability to degrade alkanes and aromatic compounds.[7] In some places the dominance of microorganisms were found, for example, in Daqing oily fields in China, the dominance of *Acidobacteria*, *Actinobacteria*, *Bacteriocytes*, *Chlorogenic*, *Planctomycetes* and *Proteobacteria.*[5] Furthermore, *Enterobacter sp*. CMG 457 was identified as a highly resentence to organ hydrocarbons like aromatic, paraffin and pesticides from oil pump in paradise habitation in Karachi city in Pakistan, in addition to their tolerant of 3mM of heavy metals like $CuSO_4$, $CdCl_2$ and $CrCl_2$.[8] Also several recorded strains of bacteria which they are *Aeromicrobium*, *Brevibacterium*, *Burkholderia, Gordonia* and *Mycobacterium* were isolated from oily contaminated soils, these bacteria shown a good ability to degrade hydrocarbons compounds, in another study, three strains of bacteria *Bacillus subtilis* SA7, *Pseudomonas aeruginosa* SA3 sp. and *Citrobacter* SB9 beside of two species of microalgae *Aphanocaps* and *Chlorella minutissimma*. The fungus also has the ability and can degrade petrol like *Amorphoteca*, *Candida, Graphium, Neosartorya*, *Pichia, Talaromyces* and *Yarrowia*.[8]

All different groups of living microorganisms such as bacteria, fungus and algae are represented in the soil and aquatic environments have an enzymatic ability for degradation of hydrocarbons.[6] Petrol usually found as a complex mixture from kinds of hydrocarbons includes aliphatic, mono-rings and multi rings alkanes, therefore studies of biodegradation are carried out on the base of bio prospecting at contaminated sites in contaminated sites.[9] In spite of the sensitivity of bacteria towards environmental conditions, but found that there are types of bacteria can be isolated from soils that have suffered from harsh environmental conditions, for example, Bacterial strains were isolated from these soils which they are *Pseudomonas aeruginosa* JX962696.1, *P. aeruginosa* KC776528.1, *P.aeruginosa* JF919950.1, *Klebsiella pneumonia* LC093517.1, *Arthrobacter* sp. GU451067.1, *Citrobacter* sp. KR063563.1, *Citrobacter amalonaticus* KC689297.1, *Enterobacter helveticus,* JN255127.1, *Cronobacter muytjensii* JN255123.1, *Citrobacter amalonaticus* KC689297.1 and *Bacillus cereus* LN890172.1 which are unexpected to be isolated from contaminated soils from various sources including seashore near a gas station and car workshops from deep of 1-2cm. results were not exaggerated because they were diagnosed molecularly and by sprinting sp73 and sp 74.[10] In some regions, the dominance of one species on other species via the tests of ribosomal acid 16S rRNA and it has been sown the dominance of *Bacillus* species like *Bacillus cereus, B. sphaericus , B. fusiformis, B. pumilus* with less bacterial appearance of *Acinetobacter junii, Pseudomonas sp*. in polluted soils with gas oil.[11] Other conditions may determine the presence of bacterial families such as acidity, alkalinity and may be implicitly specialized on some organic substances, for some individuals which belong to the families of Azotobacteriaceae, Enterobacteriaceae, and *Pseudomonas*, the ability to grow in contaminated environments with Xylene, benzene and diphenylamine at 25-45ºC and pH=5-9, therefore they can be used in industrial remediation.[12] There is a probability of four known species have the ability to grow in contaminated soils with petrol which they are *Bacillus, Micrococcus, Pseudomonas* and

Rhodococcus, these species have already grown on the apparent oil and some aromatic hydrocarbons that have to be able to extract carbon from these materials and may be due to adaptation on harsh environmental conditions.[14] Despite the toxicity of Polycyclic aromatic hydrocarbons (PAHs), but it is possible to isolate different kinds of bacteria from different contaminated sites with oil, these bacteria have the ability of growing on different hydrocarbons like diesel, benzene, lubricants, toluene, naphthalene and kerosene. An isolated strains of bacteria are often negative gram, sometimes has a very good ability to release energy from diesel, so it can be invested in bioremediation processes[14]. Some other bacterial species isolates belong to following families Pseudomonadaceae and Moraxellaceae (species *Acinetobacter*), were known well with high ability to degrade hydrocarbons, therefore they can be stimulated for the purpose of bioremediation, the recent studies showed the species of *Pseudomonas putida*, *Acinetobacter calcoaceticus* and *Acinetobacter johnsonii* have the ability to degrade naphthalene compounds and degrade related compounds with naphthalene ring, in other hand the ability of *Acinetobacter* is higher to decompose alkanes highly carbons compounds started from C18 – C 24.[15] Here we should know a little about aerobic degradation of petroleum compounds, resent studies reported the most rapid and complete remediation through degradation bath ways of the majority of organic pollutants, were passing through under aerobic conditions, An organic pollutants is an oxidative process initially are intracellular, and as well as the activation incorporation of oxygen is enzymatic key of reaction which catalyzed by oxygenases and peroxidases. The peripheral pathways of degradation convert organic pollutants step by step sequentially into intermediates of the central intermediary metabolism.[16] The Figure 1 showed to us and let us to recognize how bacteria consume hydrocarbons and which let me say organic Nitrogen (N) from Ammonia (NH^{+}_{4}) and Sulfur (S),[17] which reached contaminated soil with petrol.

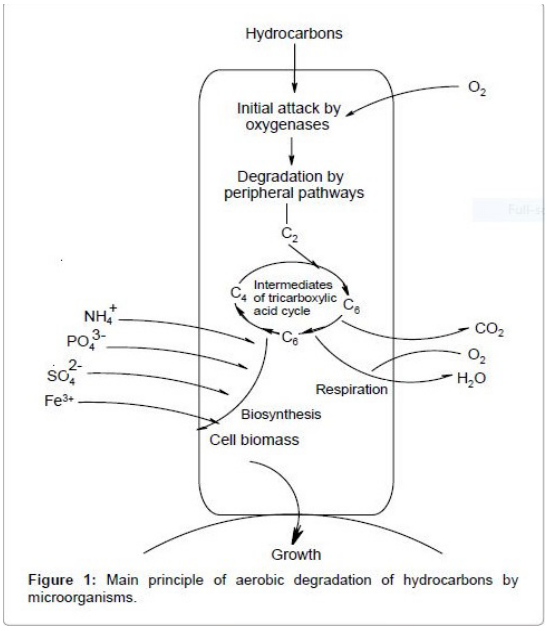

Figure 1: Main principle of aerobic degradation of hydrocarbons by microorganisms.

Figure 1 Main principle for aerobic degradation of hydrocarbons by microorganisms

Generally, bacteria in soil plays an important role in the process of environmental treatments, this study aims to identify pathogenic bacteria which found in some contaminated waters of Iraqi oil field and contaminated soils with hydrocarbons.

Materials and methods

Soil samples were being taken from Al-Sidiyeh station, brother's station in Al-Jihad neighborhood and Al-Amel station near the distribution pumps of petroleum derivatives such as diesel, gasoline and kerosene, and then transferred to the laboratory for preparation and cultivation, and samples of soil of drilling and water associated with crude oil as follows:

I. Sample of soil from Al-Ahdab field
II. Sample of soil from Al-Ahdab field
III. Water sample associated with drilling in the Al-Ahdab field
IV. Water sample associated with drilling in " "
V. Water sample associated with drilling in " "
VI. Soil sample belongs to Al-Gharaf fields in Nasiriyah Governorate

Media of soil extract agar and nutrient agar media, test tubes, sterilized water media, water bath, Petri dishes gram stains and malachite green are used to finish work

Procedure

One gm of each sample of soil are taken from surface soils of oil products stations and Al-Ahdab field were weighed, and 1 ml of water associated with crude oil samples are taken, then samples were placed in a test tube contained 9ml of sterilized distilled water in order to prepare dilution of 1/10 then two dilutions 1/100 and 1/1000 were prepared from first one, also three repetitions were prepared for each dilution. For isolation and identification of aerobic a spore former bacteria, test tubes of dilutions in a water bath at 80ºC for 20 mints to kill non-spore former vegetative cells and in order to get resistance spores which can survive at this temperature for a long time. One ml of each tube was transformed to a petri dish, and then nutrient media poured into the Petri dishes at temperature 50ºC and contents of dishes are mixed horizontally into counter-clockwise directly after poured media then left to get hard all Petri dishes are incubated at 37ºC for 72 hours, after incubation, Petri dishes are examined to test growing colonies, after that slides of growing colonies are stained via malachite green in order to identify spores and location at cells and gram stain for another slide.[18]

Results and discussions

Results of cultured soils which taken from places near generators full stations for oil products like gasoline, diesel and kerosene at Baghdad city, showed few numbers of bacteria for those samples which taken from land surface at stations of Al – Sidiyeh, brothers built in Al-Jihad Neighborhood and Al-Amiel district for calibration with oil field, a few numbers of *Bacillus* sp. bacteria are identified at contaminated sites with kerosene and diesel, but they disappeared in contaminated soils with gasoline. Either in contaminated soils with crude oil and associated waters with crude oil, results showed the different quantity of bacteria from sample to the other with the prevalence of a species of bacteria on other species, were following species were diagnosed and identified according to phenotypes and chemical tests

Pseudomonas aeruginosa

This species of bacteria were isolated from wet soil of sample No. (6) which taken from Al Gharaaf field at Al Nasiriyah.

Citation: Jessim AI, Hassan RK, Salman AS. Isolation and identification of some pathogenic bacteria species from contaminated ambient with oily hydrocarbons. *J Bacteriol Mycol Open Access*. 2020;8(4):111–115. DOI: 10.15406/jbmoa.2020.08.00282

1. **Morphology:** Bluish green bacteria has smelled like grapes smell, rod shape, motile with polar flagella, negative gram stain, can produce phycocyanin
2. **Biochemical tests:** Results showed *P. aeruginosa* was positive to some tests and negative to others as shown in Table 1.

Sphingomonas paucimobilis

This species of Bacteria is diagnosed and identified in a sample of soil No. (1 & 2) which obtained from Al-Ahdab field.

I. **Morphology:** Non-spore former bacteria, Motile rode shape and negative to gram stain and are about 1.4µm as long and 0.7µm in diameter, lives in aerobic yellow colonies, found in polluted soils and waters with organic materials

II. **Biochemical tests:** Biochemical identification showed the presence of species *S. paucimobilis* due to response for biochemical tests positively and negatively as shown in Table 2.

Bacillus subtilus

This type of bacteria is common Bactria in the environment, they are identified in drilling water of Al-Ahdab field samples No. (3&4).

a) **Morphology:** Positive to gram stain, endospore former, rod shape, and Catalase positive, they are 1 µm in long and 3µm in diameter, grew in white colonies on agar

b) **Biochemical tests:** *B. subtilis* belong to the family *Bacillaceae*, found in the dried environment and tolerate harsh conditions. *B. Subtilis* identified in water samples via biochemical tests, these tests showed positive and negative results as shown in Table 3.

Table 1 Biochemical tests of *Pseudomonas aeruginosa*

Result	Biochemical test	No.	Result	Biochemical test	No.
-	Indole	6	+	Oxidase	1
-	Methyl red	7	+	Catalase	2
+	Nitrate reduction	8	-	Fermentation	3
-	Vogas proskaur	9	-	MacConkey (lactose)	4
			+	Cetrimide agar (Blue greenish)	5

Table 2 Biochemical tests of *Sphingomonas paucimobilis*

Result	Biochemical test	No.	Result	Biochemical test	No.
+	ɣ-hymatolysis	7	+	Oxidase	1
-	Amylase	8	+	Catalase	2
+	Fermentation, glucose , lactose, dextrose, release gas, sucrose	9	-	Indole	3
	Nitrate redaction	10		Citrate	4
			-	Esculin	5
			+	H_2S in lead acetate	6

Table 3 Biochemical tests of *Bacillus subtilis*

Result	Biochemical test	No.
+	Oxidase	1
+	Amylase	2
+	Catalase	3
+	-hymatolysis β	4
+	Voges poscular	5
+	Fermentation, glucose , lactose	6
+	Motility examination	7
+	Citrate	8
+	NaCl growth 6,5%	9

Citrobacter analonaticus

Results showed the presence of this species in samples of Al-Ahdab field in samples of soil No. (1, 2 & 6) and untreated polluted associated waters.

i. **Morphology:** Rode shape bacteria, negative to gram stain and are about 2µm as long and 3 µm in diameter, anaerobic facultative living and growing in colonies

ii. **Biochemical tests:** The species of *C. amalonaticus* in the samples of waters more than soils samples as shown in biochemical tests in the Table 4.

Table 4 Biochemical tests of *Citrobacter amalonaticus*

Result	Biochemical test	No
+	Oxidase	1
+	Catalase	2
+	-hymatolysis β	3
-	Voges poscular	4
+	Fermentation, glucose , Mannose and dextrose	6
	Tyrosine arylamidase	7
+	Phosphorase	8
+	H_2S production	9
-	Mortality	10
-	Ellmar	11

Although there are a lot of a highly abundant diagnosed bacterial species, what is remarkable is the dominance of some species on other species as shown in Table 5. In this study, we found the bacterial species *S. paucimobilis* which dominated in the obtained samples of

soil from the Al-Ahdab field (1&2). As for samples of water No. 3 and 4 which taken from associated water of Al-Ahdab, results showed two dominance species *B. subtilus* and *C. amalonaticus*, these two species of bacteria known well as degradable of Bacteria,[9] but the taken sample of soil from Al-Gharaaf field results showed the dominance of *P. aeruginosa*, this is mean there are a high concentration of aromatic compounds and alkanes, *P. aeruginosa* is characterized by the good ability to degrade aromatic compounds and alkanes.[7] Therefore, bioremediation processing of soils and polluted sites with aromatic compounds and alkanes can be enhanced with *P. aeruginosa* which known as a good degradable of crude oil.[8] From the above, we found there is a difference among the identified species of bacteria in water and soil where the samples of associated water with crude oil were analyzed compared to the soil samples, it has been shown the dominance of two species of *B. subtilus* and *C. amalonaticus*, but the presence of species *S. paucimobilis* are dominant on the others in samples of Al-Ahdab field No. (1&2), this species is known in China where isolated from contaminated soils with petrol, this species of bacteria have the ability to degrade polyaromatic hydrocarbons.[19] From these results what happened to find *C. amalonaticus* formerly called *Levinea amalontica* and considered as a human pathogenic, due to its responsibility about human urinary tract infections,[20] and was identified as a genus of coliform bacteria,[21] It may belong to kind of contamination with sewage if there are a human activities near the place. For the species *S. paucimobilis* have been identified before in Iraq as I know and my colleges Rasha Kifah Hassan Saied that she identify this species in her project of bioreactor to degrade petrol contamination when she isolate it from soil and she had studied it well, however, I found from the references *S. paucimobilis* were diagnosed and identified in China before,[22] and in Iraq as a pathogenic bacteria.[23] In this case, I am wandering about this study two pathogenic species were found in soil? here there were two questions, the first one is how it be their? are they come from kind of flood come from river or from city contaminated with pathogenic resource or these species were natural flora and were moved to human body, how they changed their behaviors. If there were different conditions from heating, light, oxygen and nutrients?! How we found them in soil and they were isolated and identified from urine? I can say, may be organic nitrogen could play major role of pathogenic bacteria not for *S. paucimobilis* and *C. amalonaticus* for example some of *Bacillus* individuals like *B. cereus*. What make me believe with this idea? When I read paper[17] and found diagram of degradation path way, then also pathogenic bacteria need those organic elements which found a lot in oily contaminated soils with hydrocarbons, which lead me to suggest if we can use some of sludge from municipal sewage in bio degradation processing if it contained like these bacteria in highly number.

Table 5 Types of dominated bacteria in samples of soils and associated waters

Identified bacteria	Place of sample	Sample type	No.
Sphingomonas Paucimobilis	Al- Ahdab field	Soil	1
Sphingomonas Paucimobilis	Al- Ahdab field	Soil	2
Bacillus subtilus Citrobacter amalonaticus	Al- Ahdab field	Water	3
Bacillus subtilus Citrobacter amalonaticus	Al- Ahdab field	Water	4
Citrobacter amalonaticus	Al- Ahdab field	Water	5
Pseudomonas aeruginosa	Al-Gharaaf field	Soil	6

Recommendations

We need more and more for presence of pathogenic resources and total numbers of species and microbial load in different places before any degradation processing, or any healthy project. The qualitative and quantitative analyses of the oil and its derivative residue are required for Biodegradable Organic contaminates should be conducted to determine the success of the processing and the appropriate period of degradation for its efficiency. Soil analysis also should be carried out to determine its microbial load in order to enhance optimal nutrients if these soils which need these nutrients, also we can use sludge of municipal sewage in biodegradation to hence processing.

Acknowledgements

None.

Conflicts of Interest

The authors declare that they have no potential conflict of interests.

References

1. Thies JE, Grossman JM. *The Soil Habitat and Soil Ecology*. In Uphoff N, Ball AS, editors. 2006:66–69.
2. Killham K, Prosser JI. The bacteria and Archaea. In Paul EA, editor. *Soil microbiology, Ecology and Biochemistry*. 4th edn. 2015:41–76.
3. Masciandaro G, Macci C, Peruzzi E, et al. Organic matter–microorganism–plant in soil bioremediation: a synergic approach. *Rev Environ Sci Biotechnol*. 2013;12:399–419.
4. Lynch JM, Benedetti A, Insam H, et al. Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. *Biol Fertil Soils*. 2004;40:363–385.
5. Peng M, Zi X, Wang Q. Bacterial Community Diversity of Oil-Contaminated Soils Assessed by High Throughput Sequencing of 16S rRNA Genes. *International Journal of Environmental Research and Public Health*. 2015;12:12002–12015.
6. Jahangeer Kumar V. An Overview on microbial degradation of petroleum hydrocarbon contaminants. *International Journal of Engineering and Technical research*. 2013;1(8):34–37.
7. Mittal A, Singh P. Isolation of hydrocarbon degrading bacteria from soils contaminated with crude oil spills. *Indian Journal of Experimental Biology*. 2009;47:760–765.
8. Shahab S, Shafi I, Ahmed N. Indigenous Oil Degrading Bacteria: Isolation, Screening and Characterization. *National Journal of Health Sciences*. 2017;2:100-105.
9. Agnes MN. Screening, Isolation and Characterization of Hydrocarbonoclastic Bacteria from Oil Contaminated Soils. University of Nairobi; 2017:65.
10. Al Disi Z, Jaoua S, Al-Thani D, et al. Isolation, Screening and Activity of Hydrocarbon-Degrading Bacteria from Harsh Soils. Proceedings of the World Congress on Civil, Structural, and Environmental Engineering (CSEE'16). Paper No. ICESDP 104. Prague, Czech Republic. 2016:1–4.

11. Bento FM, Camargo FAO, Okeke BC, et al. Bioremediation of soil contaminated by diesel oil. *Brazilian Journal of Microbiology*. 2003;34(Suppl.1):65–68.

12. Athar MA, Akbar A, Khan YH, et al. Characterization of Hydrocarbon Degrading Bacteria Isolated from Hydrocarbon Contaminated Soil. *Journal of Pure and Applied Microbiology*. 2014;(6):1–8.

13. Mirdamadian SH, Emtiazi G, Golabi MH, et al. Biodegradation of Petroleum and Aromatic Hydrocarbons by Bacteria Isolated from Petroleum-Contaminated Soil. *J Pet Environ Biotechnol*. 2010;1(102):1–5.

14. Geetha SJ, Joshi SJ, Kathrotiya S. Isolation and characterization of hydrocarbon degrading bacterial isolate from oil contaminated sites. *APCBEE Procedia*. 2013;5:237–241.

15. Alavijeh PK, Halimoon N, Zakaria MP, et al. Crude Oil Biodegradation Using Isolated Bacteria from Oil Polluted Soil. In Aris AZ, Tengku Ismail TH, editors. From Sources to Solution Proceedings of the International Conference on Environmental Forensics. 2013:127–129.

16. Olajire AA, Essien JP. Aerobic Degradation of Petroleum Components by Microbial Consortia. *J Pet Environ Biotechnol*. 2014;5(5):1–22.

17. Fritsche W, Hofrichter M. Aerobic degradation by microorganisms. In: J Klein, editor. *Environmental Processes- Soil Decontamination*. Weinheim, Germany. Wiley-VCH; 2000.

18. De Vos P, Garrity GM, Jones D, et al. BERGEY'S MANUAL OF Systematic Bacteriology. 2nd edn. 2009.

19. Zhou L, Li H, Zhang Y, et al. Sphingomonas from petroleum-contaminated soils in Shenfu, China and their PAHs degradation abilities. *Brazilian Journal of Microbiology*. 2016;47(2):271–278.

20. Garcia V, Abat C, Moal V, et al. Citrobacter amalonaticus urinary-tract human infections, Marseille, France. *New Microbes and New Infections*. 2016;11:1–5.

21. Leclerc H, Mossel DAA, Edberg SC, et al. Advances in the Bacteriology of the Coliform Group: Their Suitability as Markers of Microbial Water Safety. *Annual review of microbiology*. 2001;55:201–234.

22. Yabuuchi E, Yano I, Oyaizu H, et al. Proposals of Sphingomonas paucimobilis gen. nov. and comb. nov., Sphingomonas parapaucimobilis sp. nov., Sphingomonas yanoikuyae sp. nov., Sphingomonas adhaesiva sp. nov., Sphingomonas capsulata comb. nov., and two genospecies of the genus Sphingomonas. *Microbiol Immunol*. 1990;34(2):99–119.

23. Osman A. Antibiotic Resistance of Bacteria isolated in Urinary Tract Infections in Erbil City. *Zanco Journal of Pure and Applied Sciences*. 2019;31(4):42–49.

Citation: Jessim AI, Hassan RK, Salman AS. Isolation and identification of some pathogenic bacteria species from contaminated ambient with oily hydrocarbons. *J Bacteriol Mycol Open Access*. 2020;8(4):111–115. DOI: 10.15406/jbmoa.2020.08.00282